Tree species richness increases ecosystem carbon storage in subtropical forests

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Abstract: Forest ecosystems are an integral component of the global carbon cycle as they take up and release large amounts of C over short time periods (C flux) or accumulate it over longer time periods (C stock). However, there remains uncertainty about whether and in which direction C fluxes and in particular C stocks may differ between forests of high versus low species richness. Based on a comprehensive dataset derived from field-based measurements, we tested the effect of species richness (3–20 tree species) and stand age (22–116 years) on six compartments of above- and below-ground C stocks and four components of C fluxes in subtropical forests in southeast China. Across forest stands, total C stock was 149 ± 12 Mg ha ¹ with richness explaining 28.5% and age explaining 29.4% of variation in this measure. Species-rich stands had higher C stocks and fluxes than stands with low richness; and, in addition, old stands had higher C stocks than young ones. Overall, for each additional tree species, the total C stock increased by 6.4%. Our results provide comprehensive evidence for diversity-mediated above- and below-ground C sequestration in species-rich subtropical forests in southeast China. Therefore, afforestation policies in this region and elsewhere should consider a change from the current focus on monocultures to multi-species plantations to increase C fixation and thus slow increasing atmospheric CO concentrations and global warming.

DOI: https://doi.org/10.1098/rspb.2018.1240

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Abstract

Forest ecosystems are an integral component of the global carbon cycle as they take up and release large amounts of C in short time (C flux) or accumulate it over longer time (C stock). However, there remains uncertainty about whether and in which direction C fluxes and in particular C stocks may differ between forests of high vs. low species richness. Based on a comprehensive dataset derived from field-based measurements, we tested the effect of species richness (3–20 tree species) and stand age (22–116 years) on six compartments of above- and belowground C stocks and four components of C fluxes in subtropical forests in south-east China. Across forest stands, total C stock was 149 ± 12 Mg ha^{-1} with richness explaining 28.5% and age explaining 29.4% of variation in this measure. Species-rich stands had higher C stocks and fluxes than stands with low richness; and, in addition, old stands had higher C stocks than young ones. Overall, for each additional tree species the total C stock increased by 6.4%. Our results provide comprehensive evidence for diversity-mediated above- and belowground C sequestration in species-rich subtropical forests in south-east China. Therefore, afforestation policies in this region and elsewhere should consider a change from the current focus on monocultures to multi-species plantations to increase C fixation and thus slow increasing atmospheric CO_2 concentrations and global warming.

Keywords: BEF-China, carbon storage, carbon flux, ecosystem functioning, evergreen broad-leaved forest, forest biodiversity

1. Introduction

As an integral component of the global carbon cycle, forests store about 45% of terrestrial carbon (C) and account for a net sink of 1.1 Pg C per year [1, 2]. Regulating atmospheric C concentration by preserving and enhancing forest C stocks has been recognized as a major political target to mitigate global climate change [3]. In particular, reliable benchmark estimates of forest carbon stocks and knowledge how forest C density can be promoted are required for the successful implementation of forest management strategies [4]. In this context, tree diversity, typically measured as tree species richness per area, is regarded as an important component influencing forest productivity and carbon storage at global and local scales [5, 6]. However, our knowledge about the significance of tree species richness for forest C cycling is still limited and largely concentrated on aboveground assessments [5, 7].

Global syntheses have confirmed that plant species richness in general has positive effects on plant productivity and other components of the C cycle [8, 9]. Previous research conducted in tree plantations [10, 11] and forests [5, 7, 12, 13] suggests that the results from grassland ecosystems might also apply to forest ecosystems. Several mechanisms how species richness may affect ecosystem productivity, and thereby the size of ecosystem C stocks and the balance between C gains and losses have been identified. Besides selection effects, niche complementarity and biotic and abiotic facilitation are major mechanisms underlying biodiversity–ecosystem functioning (BEF) relationships [14]. Selection effects result from the increasing chance that well-performing and dominant species driving ecosystem functions are present in diverse communities [15]. According to the niche complementarity hypothesis [16], species richness promotes resource use and nutrient retention and thus allows larger C stocks per area [17]. It has also been suggested that

variability of C stocks and fluxes should decrease with species richness, resulting in a more predictable balance between C gains and losses over longer time periods [18, 19]. Facilitation occurs when a species enhances the performance of another species in a plant community [20]. Biotic facilitation comprises for example enemy and pathogen dilution effects when trees grow better in species-rich forests due to lower encounter and transmission rates of herbivores [21] and plant pathogens [22]. In contrast, abiotic facilitation refers to an improved abiotic environment for plant growth, which can be related for example to better nutrient availability or microclimatic conditions [20].

Tree species richness has been shown to positively affect aboveground stand productivity [23], aboveground tree C storage [24], leaf litter production [25], litter decomposition [26] or soil C storage [13]. Whereas stand productivity, including leaf- and root-litter production, and tree C storage can directly be influenced by tree species richness, the size of other C stocks such as those in soil and litter are rather controlled by the balance between litter input and decomposition dynamics. There is evidence that richness has the potential to positively influence C fluxes in and out of forest ecosystems, but it is not clear if these positive effects on fluxes cancel out and leave multiple components of C stocks unchanged in their sum [27] or increase them [24]. For example, the balance could be shifted if species richness has a stronger influence on decomposition dynamics and consequently carbon loss than on C capture.

To understand how tree species richness influences total C storage, it is crucial to consider as many of the major components of the C cycle as possible. Furthermore, because many previous studies focused on intensively managed forests [10, 11], with richness levels rarely exceeding four tree species, earlier results might only be transferable to a limited extent to more species-rich forests [28, 29]. That biodiversity

112 effects on aboveground biomass are prevalent even at high richness levels has been
113 recently shown by several studies [12, 30]. In a subtropical tree biodiversity
114 experiment aboveground wood productivity was 122% higher in the most species-rich
115 communities composed of 24 tree species than in the average monoculture [31]. In
116 that analysis stand productivity increased linearly with the logarithm of species
117 richness, indicating a flattening of the strength of richness effects, possibly due to
118 increasing functional redundancy among species [32]. Weak or no
119 biodiversity–ecosystem functioning relationships have also been observed in some
120 studies in old-growth tropical forests, which suggested that these relationships can
121 also depend on spatial scale [33-35].

122 Besides species richness, stand age is often considered an even more important
123 factor driving C-cycling processes in forest ecosystems. During secondary forest
124 succession C accumulates in above- and belowground C stocks while stand
125 productivity often declines because of increased mortality and declining tree densities
126 [36]. Because age and richness may be correlated, effects of the two may be
127 confounded, such that richness effects are in part explained by age effects and the
128 other way round [32]. Furthermore, age and richness may interactively affect
129 ecosystem functioning [37]. For example, assuming low functional redundancy
130 between species in early successional forests, an increase in richness might have
131 stronger effects on productivity than in late successional forests with high functional
132 redundancy. Only by resolving the relative effects of tree species richness and stand
133 age on C-cycling processes, reasonable predictions about the role of species richness
134 during forest succession are possible.

135 Subtropical broad-leaved forests in East Asia are a hotspot of tree species
136 richness but still underrepresented in C-cycle research. Due to large afforestation

programs, forest cover in this region is increasing, thus representing an important C sink in China [38]. However, most of current afforestation focuses on monoculture plantations [39], and it remains unknown if this can compensate for previous C losses due to earlier deforestation of species-rich forest. Using a comparative study approach, we estimated the effects of species richness and stand age on multiple components of the C cycle (six components of C stocks and four components of C fluxes) and the resulting total forest C stock in a subtropical forest in south-east China. Our study included 27 forest plots established along crossed gradients in richness and age to estimate multiple components of C stocks and fluxes. We address the following overall research question: how are total and component carbon stocks and fluxes controlled by tree species richness and stand age? Specifically, we test the following hypotheses. Hypothesis H1 refers to effects of species richness: richness promotes component C stocks (H1a) and C fluxes (H1b), with a positive effect on total forest C storage (H1c). We expect that the positive effect of richness on C gain (tree aboveground C increment) outweighs richness effects on C loss (respiration, decomposition), resulting in an overall positive richness effect on forest C storage. Hypothesis H2 refers to effects of stand age: component C stocks (H2a) and C fluxes (H2b) are larger in old than in young stands, with a positive effect of stand age on total forest C storage (H2c). To conclude, we compare results of our study with C stocks of conventional single-species plantations in subtropical China to estimate potential C gains and financial assets under a multi-species planting scheme.

2. Material and methods

(a) Study site

The study was conducted within the Gutianshan National Nature Reserve (GNNR) in

Zhejiang Province, south-east China (29°140'N, 118°070'E). GNNR covers an area of about 81 km^2. The region is characterized by a warm-temperate climate with distinct seasonality [40]. A large portion of the GNNR is covered by mixed evergreen broad-leaved subtropical forest of advanced and young successional stages harboring a total of 1426 plant species belonging to 648 genera and 149 families. The most prevalent soils of GNNR are sandy-loamy and silty-loamy acidic Cambisols developed on granite or on saprolite. More site information is provided by Bruelheide *et al.* [40].

In 2008, twenty-seven plots of 30 × 30 m distributed within an area of 33 km^2 were established to assess variables related to ecosystem C-cycling along crossed gradients of species richness (3–20 tree species per plot) and stand age (22–116 years). This plot size was chosen as representative for this forest ecosystem with a canopy height of less than 30 m; that is, we considered C-cycling variables as scale-invariant above this plot size. Larger plots would have increased the precision of measurements (and thus reduced the error variation) but would not have allowed us to measure as many as 27 plots across the same total area. More importantly, because of the species–area relationship, richness is not scale-invariant and increases continually with plot size, so that correlations with ecosystem variables also change. We found these correlations to be strongest for plot sizes of 30 × 30 m and 40 × 40 m using data from a nearby 24-ha permanent forest plot [41], consistent with the assumption that this was a relevant ecosystem scale. For each plot, richness was determined as the number of species with at least one tree individual with diameter at breast height (DBH) > 10 cm, whereas stand age was determined by tree ring analysis based on the age of the fifth-largest tree in each plot [40]. The different stand ages were due to different times since the last agricultural use or tree harvesting before the Nature

187 Reserve was established [40]. Despite the current protection status, we observed that
188 in two of the 27 selected plots some trees were harvested, leading to the exclusion of
189 these two plots from some analyses (table 1).
190
191 **(b) Quantification of C stocks and fluxes**
192 To estimate C stocks (expressed in Mg C ha^{-1}), we included six components and
193 grouped them into three layers (table 1). These components were aboveground C in
194 trees (Tree_AGC), aboveground C in herbs (Herb_AGC), forest-floor litter-layer C
195 (Litter_C), deadwood C (DW_C), root C (Root_C) and soil C (SOC). The layers were
196 aboveground total C (AGC_total = Tree_AGC + Herb_AGC), ground total C
197 (GC_total = Litter_C + DW_C) and belowground total C (BGC_total = Root_C +
198 SOC). Finally, the six component stocks were summed up to an estimate of the total C
199 stock (Total_C). C fluxes (expressed in Mg C ha^{-1} yr^{-1}) were calculated from repeated
200 measurements of tree sizes, soil respiration, litter production and coarse deadwood
201 decomposition. The four components were aboveground C increment in trees
202 (AGC_F), litterfall C flux (Litterfall_F), coarse woody debris C flux (CWD_F) and
203 soil respiration (Rs) (table 1).
204 Detailed information of the field measurement and estimation method for each
205 component of C stocks and fluxes is provided in the electronic supplementary
206 material ("Material and methods").
207
208 **(c) Measurements of environmental covariates**
209 According to previous studies and the local situation, we considered elevation, slope,
210 aspect and soil pH as covariates in our study, becasue they potentially have strong
211 impacts on C cycling processes. Plot elevation ranged from 251 m to 903 m a.s.l.

212 Because aspect is a circular variable, we used cosine and sine of aspect as northness
213 and eastness, respectively. Soil pH was determined potentiometrically in a 1:2.5
214 soil-water suspension for the top 5 cm of the mineral soil. We then used a principal
215 component analysis (PCA) to extract orthogonal axes of these five environmental
216 covariates. At last, the first axis with highest loading from soil pH and elevation was
217 used to characterize environmental variation among plots for subsequent analyses.
218
219 **(d) Statistical analyses**
220 We used several approaches to explain variation in the dependent variables, C stocks
221 and fluxes, as a function of the explanatory variables, species richness, stand age and
222 environmental variation. All these analyses were based on regression. We first applied
223 hierarchical partitioning to separate the amount of variation explained by the different
224 explanatory variables when they were all considered together in multiple regression
225 (R package "hier.part" [42]). Second, we used linear regression to determine how the
226 two main explanatory variables, richness and age, affected the dependent variables
227 individually. Third, we used multiple regression with richness and age to inspect the
228 richness effects on C stocks and fluxes at different ages and to inspect the age effects
229 at different richness levels. We did not include the environmental variation in the
230 multiple regression analyses presented in the main text because its influence was
231 generally small and plots were not deliberately selected to represent environmental
232 variation (but see table S2 in the electronic supplementary material for an analysis
233 including environmental variation). To display richness effects, we plotted — along
234 with the regression lines from the linear regression — the partial regression lines from
235 the multiple regression for the three ages 37, 64 and 95 years (see figure 3, 4). These
236 are the mean ages for eight plots classified as young forests, nine plots classified as

237 medium forests and ten plots classified as old forests, respectively. A similar approach
238 was used to display age effects (see electronic supplementary material, figure S1).
239 Fourth, we tested for interactions between richness and age in multiple regression
240 analyses but because these were never significant we did not include them in the final
241 models. All values of C stocks and fluxes in all analyses except the benchmarking
242 (see figure 1) were log-transformed to meet the normality assumption. At last, we
243 used structural equation models as implemented in the R package "lavaan" [43] to
244 explore hypothesized causal relationships between variables and derive path-analytic
245 diagrams (see electronic supplementary material, figure S3). For these analyses we
246 included the first principal component (PC1) form the environmental PCA as
247 covariate. All analyses were conducted with the R statistical software [44].
248
249 **3. Results**
250 **(a) Benchmarking of C stocks**
251 Overall, the mean Total_C across all 27 plots was 149.2 Mg C ha^{-1}, with AGC_total,
252 GC_total and BGC_total contributing 57.5 Mg C ha^{-1}, 10.5 Mg C ha^{-1} and 80.7 Mg C
253 ha^{-1}, respectively (figure 1). On average, the largest amount of C was stored in the soil
254 (SOC, 61.1 Mg C ha^{-1}), followed by Tree_AGC (57.4 Mg C ha^{-1}, figure 1). The other
255 C stocks ranking after these two were Root_C (18.3 Mg C ha^{-1}) > DW_C (6.5 Mg C
256 ha^{-1}) > Litter_C (4.0 Mg C ha^{-1}) > Herb_AGC (0.1 Mg C ha^{-1}). More C was generally
257 stored below than above ground (figure 1).
258

(b) Hierarchical partitioning of variation in C stocks and fluxes due to species richness, stand age and environmental variation

The amount of variation in each C component, C layer, total C and C flux explained by species richness, stand age and environmental variation is shown in figure 2 and table S1 in the electronic supplementary material. For C components, richness played a strong role in explaining variation in Tree_AGC, DW_C and Root_C, but played a lesser role in Herb_AGC and SOC. In comparison with richness, age accounted for a slightly higher proportion of variation in these components. For Total_C, richness explained about the same proportion (28.5%) of variation as age did (29.4%). Overall, richness and age together explained around 50% of the variation in all carbon stocks except Herb_AGC, SOC and BGC_total. The variation attributed to the environment was generally low except for SOC and BGC_total (ranging from 1.7% to 31.6%). With regard to carbon fluxes, the effects of richness and age were both large and similar for Litter_F (20.2% vs 28.7%) and CWD_F (24.3% vs 24.8%). However, richness and age could only explain a small proportion in the variation of the productivity-related component fluxes AGC_F and Rs. The environment explained from 1.4% to 11.6% of the variation in component C fluxes.

(c) Effects of species richness and stand age on C stocks (H1a, c and 2a, c)

All components of C stocks except Herb_AGC increased significantly with increasing species richness (H1a, figure 3a). Likewise, C stocks were larger in older than in younger stands except for Herb_AGC and SOC (H2a, electronic supplementary material, figure S1a), which showed marginally negative and marginally positive trends with age, respectively. Estimated AGC_total, GC_total, BGC_total and, in particular, Total_C were larger in more species-rich and older compared with less

284 species-rich and younger stands (figure 3b and electronic supplementary material,
285 figure S1b; H1c and H2c). Partial regressions predicted that the relationship between
286 richness and C stocks was positive for given age levels (figure 3b), suggesting an
287 independent positive effect of richness increasing C stocks in young, medium and old
288 forests.
289
290 **(d) Effects of species richness and stand age on C fluxes (H1b and 2b)**
291 Two of the C fluxes, Litterfall_F and CWD_F, increased significantly with increasing
292 species richness (H1b) and these effects were consistently positive across stand ages
293 (figure 4). Although AGC_F and Rs did not significantly change with richness in
294 simple linear regressions, the partial regressions predicted increasing C flux within
295 given age levels with increasing richness (figure 4). This result was due to the
296 negative effect of age on AGC_F and Rs (electronic supplementary material, figure
297 S1c), which counterbalanced the positive effect of richness in the simple linear
298 regression. The other two components of C fluxes, Litterfall_F and CWD_F, were
299 larger in older than in younger stands.
300
301 **(e) Structural equation modeling**
302 With the structural equation models using the overall correlation matrix between
303 variables (electronic supplementary material, figure S2), we first found that AGC_F
304 was indeed influenced by all three explanatory variables, but with a negative effect of
305 age balancing the positive effect of richness (electronic supplementary material, figure
306 S3a). AGC_F then increased Tree_AGC, but richness still had an additional strongly
307 positive effect on Tree_AGC, indicating that the total richness effect was only partly
308 mediated by the indirect effect via AGC_F (electronic supplementary material, figure

309 S3b). Richness also increased Root_C, which was correlated with Tree_AGC. In
310 contrast to these direct effects, the significant effects of richness on litterfall and
311 deadwood variables found in the regression analyses mentioned above appeared to be
312 indirect, i.e. working via the increased Tree_AGC which in turn promoted litterfall
313 and deadwood variables (electronic supplementary material, figure S3c, d). For SOC
314 and Rs, we could not find good explanations in the form of path analyses, indicating
315 that these were affected by other unmeasured variables. In the case of SOC the
316 influence of environmental variation was particularly strong (see figure 2).
317

318 **4. Discussion**

319 Total ecosystem C in the subtropical forest studied here was 149 Mg C ha^{-1} across all
320 forest stands. Similar or higher C stocks have been reported from other subtropical
321 forests in China [45, 46]. Across all forest stands we found that most C was stored in
322 tree standing biomass with tree aboveground and root C contributing 57 Mg C ha^{-1}
323 and 18 Mg C ha^{-1}, respectively. Soil as the second largest C stock after tree biomass
324 accounted for 41% of total ecosystem C stock, which was similar to other studies [47].
325 Whereas previous studies have focused on aboveground tree biomass or soil C storage,
326 our study demonstrates that roots, ground litter (4 Mg C ha^{-1}) and deadwood (6.5 Mg
327 C ha^{-1}) represent major components of C stocks and thus should not be neglected in
328 forest inventories [48].
329

330 **(a) Effects of species richness on C storage**

331 Our research underlines the importance of tree species richness as a driver of the C
332 cycle in the studied subtropical forest and provides comprehensive evidence for
333 diversity-mediated C sequestration above and below ground. Using a study design

with plots selected to represent a range of species richness levels and stand ages, we found that higher richness was associated not only with larger C stocks (H1a) and C fluxes (H1b) in these stands but also with larger total C storage (H1c). To a certain extent these richness effects were correlated with effects of age, because these two explanatory variables were not fully orthogonal. Thus, partial regression coefficients, i.e. effects of richness for plots of given age, were usually somewhat smaller than linear regression coefficients, except for two components of C fluxes (AGC_F and Rs), where the opposite was the case (see figure 3, 4). Beyond this, the richness effects on C stocks and fluxes did not change with age, that is none of the interactions between these two explanatory variables were significant, indicating that richness and age had additive effects on C stocks and fluxes. Previous studies using sample-survey designs, that is with most plots having intermediate richness, also found positive relationships with measures of aboveground forest productivity [5, 23].

As summarized by Poorter *et al.*, tree species richness could promote higher stand productivity and thus higher accumulated tree C stocks by niche complementarity or sampling-/selection-/insurance-type effects [12]. For our study site, there is evidence that higher richness allows both a higher stem density and larger tree sizes [49], and thus higher tree aboveground C stocks per unit area, probably as a result of reduced interspecific competition due to complementary resource use between species [47]. Complementary resource use between tree species may be related to belowground root complementarity, as observed in a manipulative biodiversity experiment near the field site of the present study [50], or to aboveground crown complementarity allowing higher canopy packing and greater light interception in more diverse forest stands [51].

We showed that higher richness promotes C stocks of deadwood and the litter layer, which is probably a result of a generally higher tree biomass translating into more litter and deadwood. Among the components of C stocks that we studied, only the herb layer (Herb_AGC) was negatively related to tree species richness (figure 3a), which was likely caused by increased competition from trees [52].

Although AFG_F and soil respiration increased with richness for plots of given age (figure 4), the linear regressions of these two dependent variables against richness were not significant because these components of C fluxes were smaller in older than in younger stands (electronic supplementary material, figure S1c) and there were more species-rich old than young stands among the 27 study plots. Overall, as shown by the results of path analyses, the positive effect of richness and the negative effect of age on AFG_F were nearly counterbalanced (see figure S3a). This suggests that increased richness may slow down or even prevent declines of stand AFG_F in older forest. Autotrophic respiration by roots can be a major contributor to soil respiration, thus allowing richness to affect soil respiration by increased root biomass and productivity [53]. The significantly higher C fluxes via litter production and deadwood decomposition in more species-rich plots were probably the result of diversity-promoted C stocks in living and dead biomass as indicated by path analyses (see figure S3c, d).

Although we could not relate tree species richness to whole-ecosystem productivity because we did not account for belowground productivity (root growth, root exudates), our data indicate that richness promoted a positive balance between C gains and losses over time. It has previously been argued that richness might primarily affect C fluxes, whereas the total C stock would be less responsive [27]. In this perspective, increased C uptake would be compensated by higher respiratory C loss,

383 thereby leaving ecosystem C storage unchanged. In contrast, our results indicate that
384 richness effects on C stocks can be stronger than richness effects on C fluxes. Thus,
385 our results suggest that for the studied subtropical forests, richness does not simply
386 speed up the C cycle but also allows the forests to retain a larger amount of C in C
387 storage.
388
389 **(b) Effects of stand age on C storage**
390 Accumulations of C stocks with forest age are well studied [54], even though rarely
391 corrected for correlated effects of increasing species richness with increasing age. In
392 our study we analyzed both overall (linear regressions) and richness-corrected (partial
393 regression coefficients) effects of forest age on C stocks (H2a), C fluxes (H2b) and
394 the total C accumulation (H2c) and found that in part the effects of stand age were
395 correlated with effects of richness, again because these two explanatory variables
396 were not fully orthogonal (see electronic supplementary material, figure S1). Our
397 plots represented a chronosequence from 22- to 116-year old stands. Obviously,
398 forests could potentially get much older, but no older forests could be found in the
399 study region due to past agricultural land use. Over the observed forest-age gradient,
400 C stocks in live trees (Tree AGC and Root_C) were 122% larger in the oldest stands
401 than in the youngest ones, and soil C was 72% larger, strongly suggesting that these C
402 stocks increase as a forest ages. Large C accumulation in regenerating forests have
403 also been found in other studies [45, 54], underlining the large C storage potential of
404 subtropical forests in China. For example, total ecosystem C increased from 101.4 Mg
405 C ha^{-1} in a young (18 years) to 260.2 Mg C ha^{-1} in a 60-years old subtropical forest
406 [46].

Tang *et al.* found that young forests allocate assimilated C predominantly to tree biomass, whereas in old forests soil is the most important C stock [55]. This is in contrast to our study, where the larger total C stocks in older stands were underpinned by stronger age-effects on component stocks in tree biomass than in soil, although all components except Herb_AGC were larger in older than in younger stands (see electronic supplementary material, figure S1). It is conceivable that with further increases in age a similar change as the one described by Tang *et al.* could occur in our forests [55]. This would be expected due to the observed negative effect of age on aboveground primary productivity (AGC_F) combined with positive effects on ground litter (Litterfall_F) and deadwood decomposition (CWD_F).

5. Conclusions

Previous biodiversity–productivity studies in forest ecosystems reported positive effects of tree species richness on aboveground components of primary productivity, suggesting that more C might be stored in species-rich forests. We found more C below than above ground in our subtropical forests, raising the question whether richness effects might be changed if total rather than only aboveground C storage is considered. Our findings demonstrate that indeed total above- and belowground C storage in subtropical forest increases with species richness if major components of C fluxes and C stocks are considered. At the regional scale, subtropical forests in south-east China are a major potential C sink because of the implementation of large afforestation and plantation programs in the last 36 years. However, these plantations were mainly established with single species and even though they will continue to accumulate C if their continued growth can be maintained into the future, much larger

amounts of C could have been stored according to our study if plantation programs would have adopted multi-species planting schemes.

Assuming a total amount of 30.3 Tg C stored per year by the planted monoculture forests in China [56], tentatively valued at 0.4 billion dollar, and extrapolating our results to other types of forests, an additional 6.4% C ha^{-1} could have been stored per year for every additional species. As a consequence, amounts in the order of 0.3 billion dollars per year (0.4×0.064^9) might have been gained by using 10-species mixtures instead of monocultures for the 0.89×10^6 ha of planted forests in China. This assumes that the relationship between monocultures and 10-species mixtures has a similar slope as the one we found here between 3- and 20-species mixtures. At the same time, this indicates the potential gains that could still be realized by switching to species-rich plantation schemes now. Such plantation schemes may be costly to carry out but could have the additional advantage that species-rich forests can be expected to be more stable and resistant to global change and to extreme events; and they contribute to biodiversity maintenance. Therefore, biodiversity should be considered as an integral component in forest-carbon management strategies.

Ethics statement

This research was performed in accordance with the laws, guidelines and ethical standards of China, Switzerland and the European Union, where the research was performed.

Data availability

The data supporting the findings of this study are deposited in Dryad with the accession code doi:10.5061/dryad.t9t0sc4.

Authors' contributions

X.L., S.T., B.S. and K.P. designed the study, X.L., S.T., J-S.H., H.B., Z.T., A.E., M.S., K.A.P., B.Y., P.K., T.S., Y.H., C.W., M.S., K.S. and P.A.N. conducted the measurements and contributed data, X.L., S.T. and B.S. conducted the analyses and wrote the manuscript, with help from all other co-authors.

Competing interests

We have no competing interests.

Funding

This study was financially supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (XDB31000000), the National Science and Technology Ministry Major Project (2017YFA0605103), the National Key Project for Basic Research Program of China (No. 2014CB954100), the German Research Foundation (DFG FOR 891), the Swiss National Science Foundation (SNSF No. 130720, 147092) and the University of Zurich Research Priority Program on Global Change and Biodiversity (URPP GCB).

Acknowledgments

We acknowledge the support of the BEF-China research group, especially many students and local helpers involved in setting up the plots and collecting the numerous data. We also thank the local support from the administration Bureau of the Gutianshan National Nature Reserve. We thank Norma Fowler, Lourens Poorter and an anonymous reviewer for their extensive and very helpful comments about earlier

481 versions of this paper.

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641 **Table 1.** Definitions of variables of C stocks and fluxes. Particularly time-consuming measurements (Root_C and Rs) were assessed on a subset

642 of plots; in two plots Tree_AGC, AGC_F and derived variables could not be assessed because farmers harvested some trees.

	Abbreviation	Definition	Measuring period	Number of repeated measures	Number of plots
C stocks ($Mg\ C\ ha^{-1}$)	Tree_AGC	Aboveground tree C	2008	1	25
	Herb_AGC	Aboveground herb C	2008	1	27
	Litter_C	Litter-layer C	2009-2010	4	27
	DW_C	Deadwood C	2009	1	27
	Root_C	Root C	2008-2009	1	15
	SOC	Soil C	2008	1	27
	AGC_total	Total aboveground C (Tree_AGC+Herb_AGC)	2008	1	25
	GC_total	Total ground-layer C (Litter_C+DW_C)	2009-2010	1	27
	BGC_total	Total belowground C (Coarseroot C*+SOC)	2008	1	25
	Total_C	Total C stock	2008-2010	1	25
C fluxes ($Mg\ C\ ha^{-1}\ yr^{-1}$)	AGC_F	Aboveground tree C increment	2008-2010	2	25
	Litterfall_F	Total litterfall flux	2009-2014	6	27
	CWD_F	Coarse woody debris flux	2009-2015	2	27
	Rs	Soil respiration	2009-2012	4	16

643 * Here the BGC total only included coarse-root C, not fine roots and herb roots, to maximize the number of plots for the belowground

644 calculation.

Figure Legends

Figure 1. Benchmarking map of carbon stocks (solid white boxes) and fluxes (dashed grey boxes) averaged across all 27 forest stands. Numbers represent means and standard errors. Different colors of arrows indicate effects of tree species richness (red) and stand age (blue). Directions of arrows show positive (upward) and negative (downward) relationships of richness and age with respective C stocks and fluxes. No arrows are shown when effects were not significant.

Figure 2. Hierarchical partitioning of the variation explained for each component and layer of carbon stocks and fluxes by species richness, stand age and environmental variation. The term for environmental variation was obtained as principal component axis (environmental PC1) of an ordination that incorporated elevation, slope, eastness of aspect, northness of aspect and soil pH at the 27 plots.

Figure 3. Relationships between C stocks and tree species richness for (a) six components and (b) three layers and total C. Each dot represents a plot. Significant R^2 values from linear regressions are shown (***: $p<0.001$, **: $p<0.01$, *: $p<0.05$). Partial regression lines for three levels of stand ages (young, medium, old) are shown in different colors. Logarithmic scales are used for all dependent variables (y axes).

Figure 4. Relationships between the four C fluxes and tree species richness. Each dot represents a plot. Significant R^2 values from linear regressions are shown (***: $p<0.001$, **: $p<0.01$). Partial regression lines for three levels of stand ages (young, medium, old) are shown in different colors. Logarithmic scales are used for all dependent variables (y axes).

Figure 1

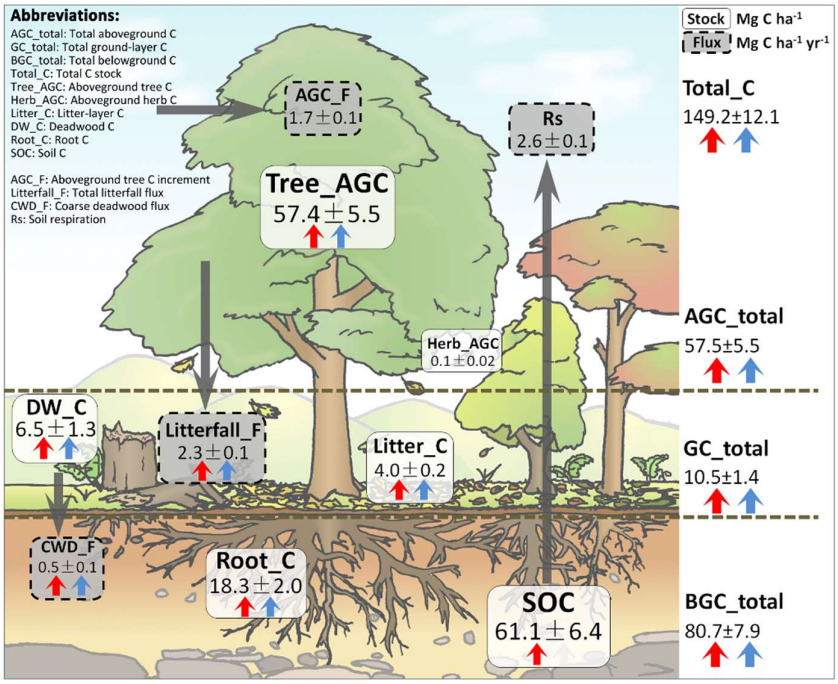

Abbreviations:
AGC_total: Total aboveground C
GC_total: Total ground-layer C
BGC_total: Total belowground C
Total_C: Total C stock
Tree_AGC: Aboveground tree C
Herb_AGC: Aboveground herb C
Litter_C: Litter-layer C
DW_C: Deadwood C
Root_C: Root C
SOC: Soil C
AGC_F: Aboveground tree C increment
Litterfall_F: Total litterfall flux
CWD_F: Coarse deadwood flux
Rs: Soil respiration
Stock Mg C ha-1
Flux Mg C ha-1 yr-1
AGC_F
1.7±0.1
Rs
2.6±0.1
Tree_AGC
57.4±5.5
Herb_AGC
0.1±0.02
DW_C
6.5±1.3
Litterfall_F
2.3±0.1
Litter_C
4.0±0.2
CWD_F
0.5±0.1
Root_C
18.3±2.0
SOC
61.1±6.4
Total_C
149.2±12.1
AGC_total
57.5±5.5
GC_total
10.5±1.4
BGC_total
80.7±7.9

Figure 2

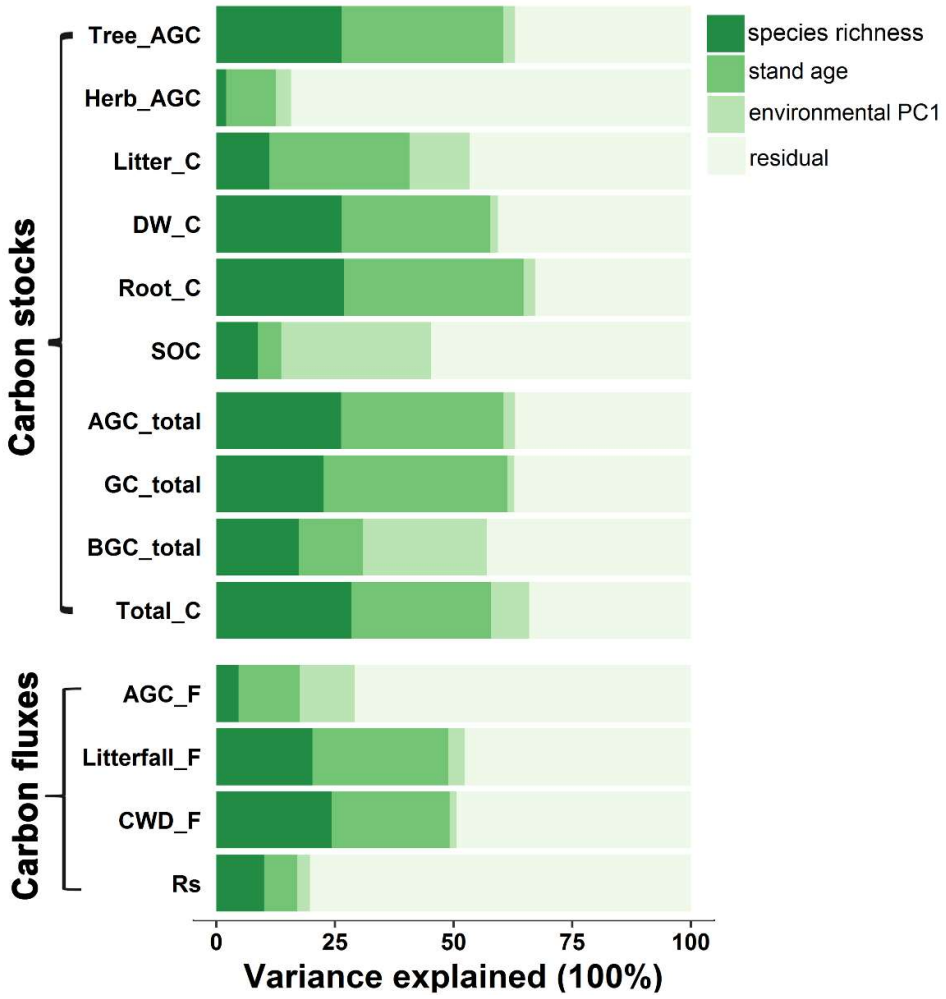

Tree_AGC
Herb_AGC
Litter_C
DW_C
Root_C
SOC
AGC_total
GC_total
BGC_total
Total_C
Carbon stocks
AGC_F
Litterfall_F
CWD_F
Rs
Carbon fluxes
species richness
stand age
environmental PC1
residual
0
25
50
75
100
Variance explained (100%)

Figure 3

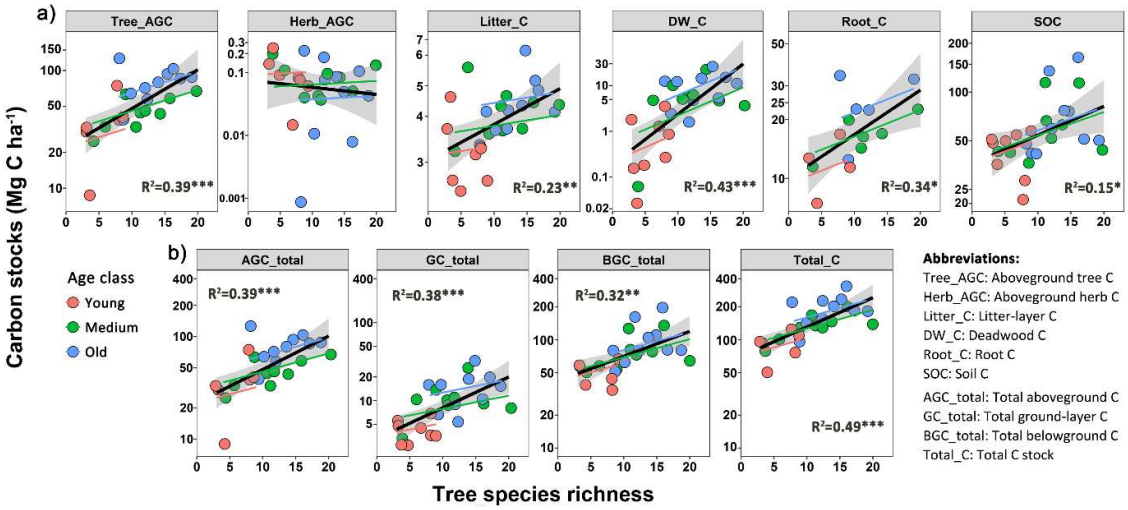
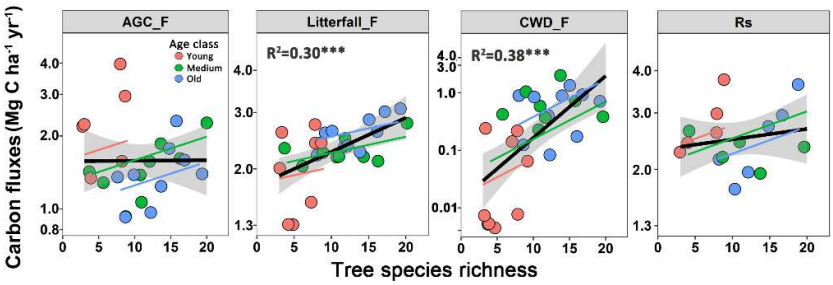

a)
Tree_AGC
Herb_AGC
Litter_C
DW_C
Root_C
SOC
R²=0.39***
R²=0.23**
R²=0.43***
R²=0.34*
R²=0.15*
b)
AGC_total
GC_total
BGC_total
Total_C
R²=0.39***
R²=0.38***
R²=0.32**
R²=0.49***
Carbon stocks (Mg C ha⁻¹)
Tree species richness
Age class
Young
Medium
Old
Abbreviations:
Tree_AGC: Aboveground tree C
Herb_AGC: Aboveground herb C
Litter_C: Litter-layer C
DW_C: Deadwood C
Root_C: Root C
SOC: Soil C
AGC_total: Total aboveground C
GC_total: Total ground-layer C
BGC_total: Total belowground C
Total_C: Total C stock

Figure 4


AGC_F
Litterfall_F
CWD_F
Rs
Age class
Young
Medium
Old
R²=0.30***
R²=0.38***
Carbon fluxes (Mg C ha⁻¹ yr⁻¹)
Tree species richness

Supplementary material

Tree species richness increases ecosystem carbon storage in subtropical forests

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Material and methods

Full methods of the field measurement and estimation of each component of C stocks and fluxes.

Quantification of C stocks

Tree_AGC — The dry aboveground biomass for each individual tree with DBH > 3 cm was estimated from DBH and height measurements using allometric equations. These allometric equations were developed with field data from the same climatic zone and vegetation type [1, 2]. The calculated biomass included dry mass of stem, branches and leaves. For the 13 most dominant species, we used species-specific equations. For the other 80 broadleaved species we used a generic allometric equation [1, 2]. If a tree had multiple stems, we estimated aboveground biomass values for each stem separately and summed them up. DBH and height for all trees were measured in 2008 when the plots were established. Tree aboveground biomass was converted to tree aboveground C by assuming a 46% fraction of C in biomass for all species [3, 4].

Herb_AGC — Aboveground biomass of the herb layer was determined at the peak of the growing season between May and July in 2008 [5]. The herb layer was defined as all plants not exceeding 1 m in height, including herbaceous and woody vascular species. Due to a large amount of leaf litter, forest-floor bryophytes were mostly absent and therefore ignored. In each plot, aboveground herb-layer biomass was harvested in four 1-m^2 sized quadrats located at the corners of the 10 x 10 m central subplot. Fresh biomass was oven-dried at 70 °C for 48 h. To convert dried biomass into C stock we assumed a C concentration of 46% of the aboveground herb layer [6].

Litter_C —To determine the biomass of the litter layer, including "all dead, fresh or dry, and partially decomposed plant tissues above the organic and mineral soil surface, including twigs up to 0.6 cm diameter" [7], we took four sample cores with a PVC tube (19 cm diameter) in undisturbed litter patches and recorded the thickness of the litter layer in the cores in all 27 plots in spring, summer, autumn and winter from 2009 to 2010. Pooled litter samples per plot were dried at 70 °C to constant weight, and C concentration was determined with an Elemental Analyzer (PE 2400 II CHN Elemental Analyzer, Perkin-Elmer, Boston, MA, USA). Litter-layer C stocks were corrected for spatial variability by using the mean of 12 additional measurements of leaf-litter thickness distributed equally along the border of each plot. To account for seasonal variation, we used the mean of litter-layer C stock averaged across seasons.

DW_C — The deadwood C stock was inventoried in all 27 plots in 2009. Deadwood comprised coarse woody debris (diameter > 10 cm) and fine woody debris

(3 cm ≤ diameter ≤ 10 cm). Lying as well as standing woody debris was recorded. Every dead tree with its base inside the plot boundaries was considered, irrespective of whether its treetop was located outside the plot area. Trees with the base outside the plot area were not considered irrespective if the top was located inside the plot. Each deadwood piece was identified to species and assigned into one of three decay classes: undecayed solid wood with bark or bark starting to fall off (class I), partly decayed wood into which a knife can be pushed by hand (class II) and strongly decayed wood that can be easily fragmented by hand (class III). Species-specific wood density was used to convert frustum volume into biomass for class I deadwood pieces. For the other decay classes (class II and III) we estimated the reduction in wood density for broadleaved and conifer species separately by sampling a representative number of wood samples with a drilling machine. Biomass of entire dead trees was calculated by allometric equations using decay-class specific wood-density data [8]. A factor of 0.45 was used to convert deadwood biomass to DW_C [4].

Root_C — Root C was calculated as the sum of C stored in tree coarse roots (diameter ≥ 2 mm), tree fine roots (diameter < 2 mm) and herb roots. Dry biomass of coarse roots for each tree was determined by allometric equations from previous studies conducted at the same field site [1]. Species-specific allometric equations (based on DBH) were used for four species (*Pinus massoniana, Cunninghamia lanceolata, Castanopsis eyrei* and *Schima superba*). A common equation with DBH and species-specific wood density was used for all the other broadleaved species [1]. Species-specific wood density was measured in the plots or taken from previous studies in the same nature reserve [9]. For species with no record of wood density, we used genus- or family-level mean values. Ten soil cores with a diameter of 10 cm and a depth of 45 cm were taken from the central point of each 10 × 10 m subplot in 15 plots in 2009 to determine fine- and herb-root biomass. After sieving and washing away the soil, live tree fine roots and herb roots were oven-dried to a constant weight. Root_C content was estimated as 44% of total biomass of coarse, fine and herb roots [3, 4].

SOC — In each plot, nine soil cores (3 cm in diameter, one per 10 × 10 m subplot), were taken to a depth of 50 cm in 2008. Soil cores were bulked to six depth increments (up to 50 cm), resulting in six soil samples for each plot. Sample preparation included hand sorting of coarse plant and animal residuals, sieving (< 2 mm) and grinding of air-dried soil samples. Soil total C content was measured by an Elemental Analyzer (Vario EL III, Elementar, Hanau, Germany). Given the acidic soil conditions in the study area, inorganic C does not occur. Therefore, measured total soil C represents the soil organic C content. SOC stocks to a depth of 50 cm were calculated according to the following equation (see [10]):

$$SOC_{stocks} = \sum_{i=1}^{n} (Depth_i \times SOC_{cont} \times BD \times (1 - (CM/100)))/100$$

where Depth is a specific depth increment (m), SOC_{cont} represents the soil organic C content related to the increment, BD is the bulk density recalculated as weighted mean based on soil horizon profile data, CM (%) is the fraction of coarse material, estimated following the German guidelines for soil description [11].

Quantification of C fluxes

AGC_F — AGC_F was calculated as the annual mean C increment of the forest stands estimated for trees with DBH > 3 cm. As for Tree_AGC, the dry biomass for

year 2008 and 2010 was first determined by allometric equations. The annual biomass increase was then converted to annual C flux by multiplication with a coefficient of 0.46 [3, 4].

Litterfall_F — Litter was collected monthly from 2009 to 2014, using five traps set in and around the central 10 × 10 m subplot in each plot. Each litter trap had an area of 0.75 × 0.75 m with 1-mm nylon mesh. The top of the trap was 1.5 m above the ground. All leaves, twigs (diameter < 2.5 cm), flowers, seeds, fruits and other reproductive parts of plants were collected monthly from traps and oven-dried at 80°C to a constant weight. Annual mean litterfall biomass was multiplied by 0.44 to estimate the Litterfall_F [4].

CWD_F — Deadwood C flux of coarse woody debris was determined as the difference in CWD C stores between two deadwood inventories in 2009 and 2014. Labeled CWD pieces in 2009 were re-measured in 2014 with transition in decay class recorded. CWD that could not be recovered in 2014 due to fragmentation or downhill movement was included as C efflux from the plot CWD pool.

Rs — Soil respiration was measured in 16 plots with a closed chamber connected to a portable infrared gas analyzer (LI-8100; Li-Cor Inc., Lincoln, NE, USA). In each plot, six PVC collars with 20 cm diameter and 8 cm height were evenly installed in 2009. The collars were inserted into the soil to a depth of 3 cm. The Rs measures were first obtained in April 2009 and remeasured every two months until March 2012. Each time, the collars were measured twice with a 1-h interval. Besides, in each season one plot was chosen to record Rs continuously over a 24-h period at 2-h intervals. Rs was calculated using the exponential equation given by [12],

$$Rs = R_{10} e^{E_0\left(\frac{1}{10-T_0} - \frac{1}{T-T_0}\right)} = R_{10} e^{E_0\left(\frac{1}{56.02} - \frac{1}{T+46.02}\right)}$$

Where R_{10} was Rs at 10 °C, T_0 and E_0 were used to define the temperature dependency of soil respiration, T was soil temperature at 5 cm depth when measuring the Rs. To facilitate the convergence of the non-linear regression procedure that was used to fit R_{10}, T_0 and E_0, we first fixed T_0 to –46.02 °C (see [62]). The total Rs was a cumulative value of hourly mean Rs of the replicated collars (n=3–6).

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164

Table S1. Percentage (%) variation in dependent variables (rows) explained by the explanatory variables tree species richness, stand age and environmental variation (first principal component axis from environmental PCA) in hierarchical partitioning analysis (see "Material and methods" in main text).

	Species richness	Stand age	Environmental PC1	Residual	Total
C stocks					
Tree AGC	26.3	34.1	2.5	37.1	100
Herb AGC	2.1	10.5	3.2	84.3	100
Litter_C	11.2	29.5	12.8	46.6	100
DW_C	26.3	31.3	1.7	40.7	100
Root_C	26.9	37.8	2.4	32.8	100
SOC	8.7	5.0	31.6	54.8	100
AGC total	26.3	34.2	2.5	37.0	100
GC total	22.6	38.7	1.5	37.2	100
BGC total	17.4	13.5	26.1	43.0	100
Total C	28.5	29.4	8.0	34.1	100
C fluxes					
AGC_F	4.7	12.8	11.6	70.9	100
Litterfall_F	20.2	28.7	3.5	47.7	100
CWD_F	24.3	24.8	1.4	49.5	100
Rs	10.0	7.0	2.7	80.3	100

Table S2. Partial regression coefficients from multiple regression analyses including the first two principal component axes from the environmental PCA analysis (environmental PC1 and PC2), stand age and species richness as explanatory variables and six C stocks and four C fluxes as dependent variables. Partial coefficients are adjusted for all explanatory variables in the multiple regression model; variation that could be explained by the joint action of two or more explanatory variables is not captured by these coefficients. Hierarchical partitioning analysis showed that environmental variation only explained a small amount of total variation in dependent variables and therefore the environmental PCs were excluded in the models presented in the main text.

	Explanatory variables											
	Environmental PC1			**Environmental PC2**			**Stand age**			**Species richness**		
Dependent variables	**regression coefficient**	**s.e.**		**regression coefficient**	**s.e.**		**regression coefficient**	**s.e.**		**regression coefficient**	**s.e.**	
C stocks:												
Tree_AGC	0.093	0.061		0.060	0.065		0.012	0.004	**	0.049	0.020	*
Herb_AGC	-0.202	0.164	.	-0.576	0.167	**	-0.017	0.010	.	0.002	0.009	
Litter_C	-0.051	0.027	.	0.013	0.027		0.005	0.002	**	0.002	0.009	
DW_C	0.095	0.184		0.462	0.187	*	0.033	0.011	**	0.146	0.061	*
Root_C	0.061	0.067		0.045	0.074		0.008	0.004	*	0.036	0.018	.
SOC	-0.197	0.060	**	0.058	0.061		0.002	0.004		0.015	0.020	
C fluxes:												
AGC_F	0.105	0.058	.	0.013	0.061		-0.007	0.004	.	0.032	0.019	
Litterfall_F	0.044	0.023	.	-0.052	0.023	*	0.004	0.001	**	0.016	0.008	*
CWD_F	0.109	0.209		0.524	0.213	*	0.028	0.012	*	0.155	0.069	*
Rs	0.068	0.072		-0.032	0.062		-0.003	0.003		0.024	0.015	

*All dependent variables were log-transformed. Significance: **: $p<0.01$; *: $p<0.05$; . : $p<0.1$

Figure S1. Relationships between stand age and C stocks and fluxes, including for (a) six components, (b) three layers and total C and (c) four fluxes. Each dot represents a plot. Significant R^2 values from linear regressions are shown (***: p<0.001, **: p<0.01, *: p<0.05). Partial regression lines for three species richness levels at 6, 11 and 16 species/plot are shown in different colors. These are the mean tree species richness levels for 10 plots classified as low, 9 plots classified as medium and eight plots classified as high species richness, respectively. Logarithmic scales are used for all dependent variables (y axes).

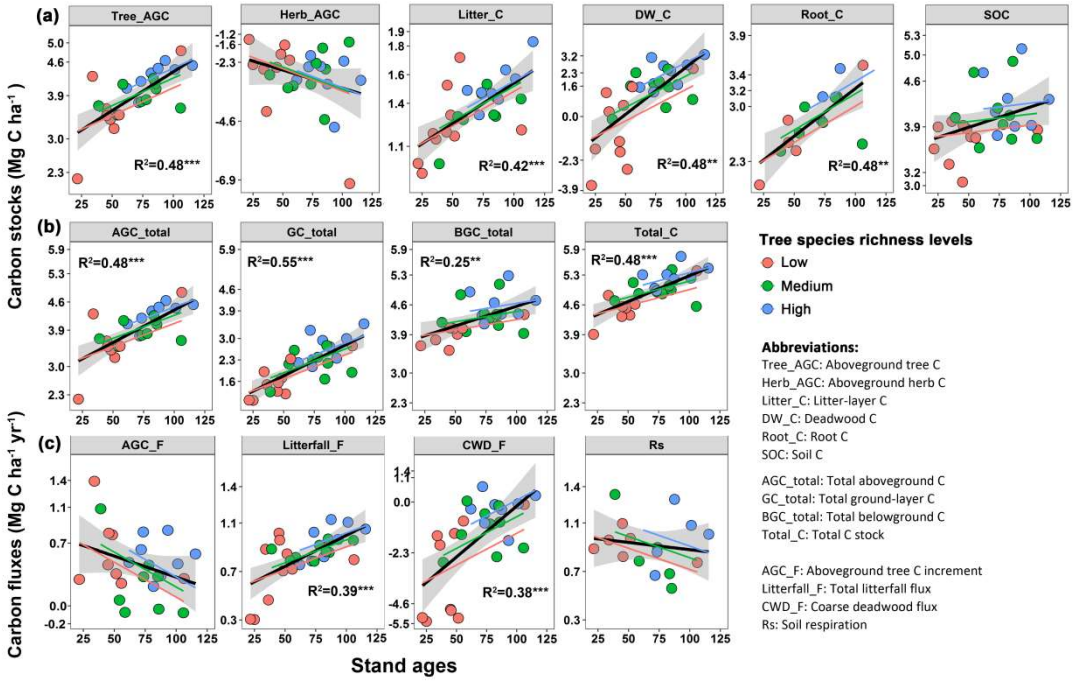

Figure S2. Correlation matrix between tree species richness, stand age, environmental PC1 ("Env"), six C stocks and four C fluxes. The absolute values of correlation coefficients r are shown in the upper triangle of the matrix. All except the first three variables were log-transformed.

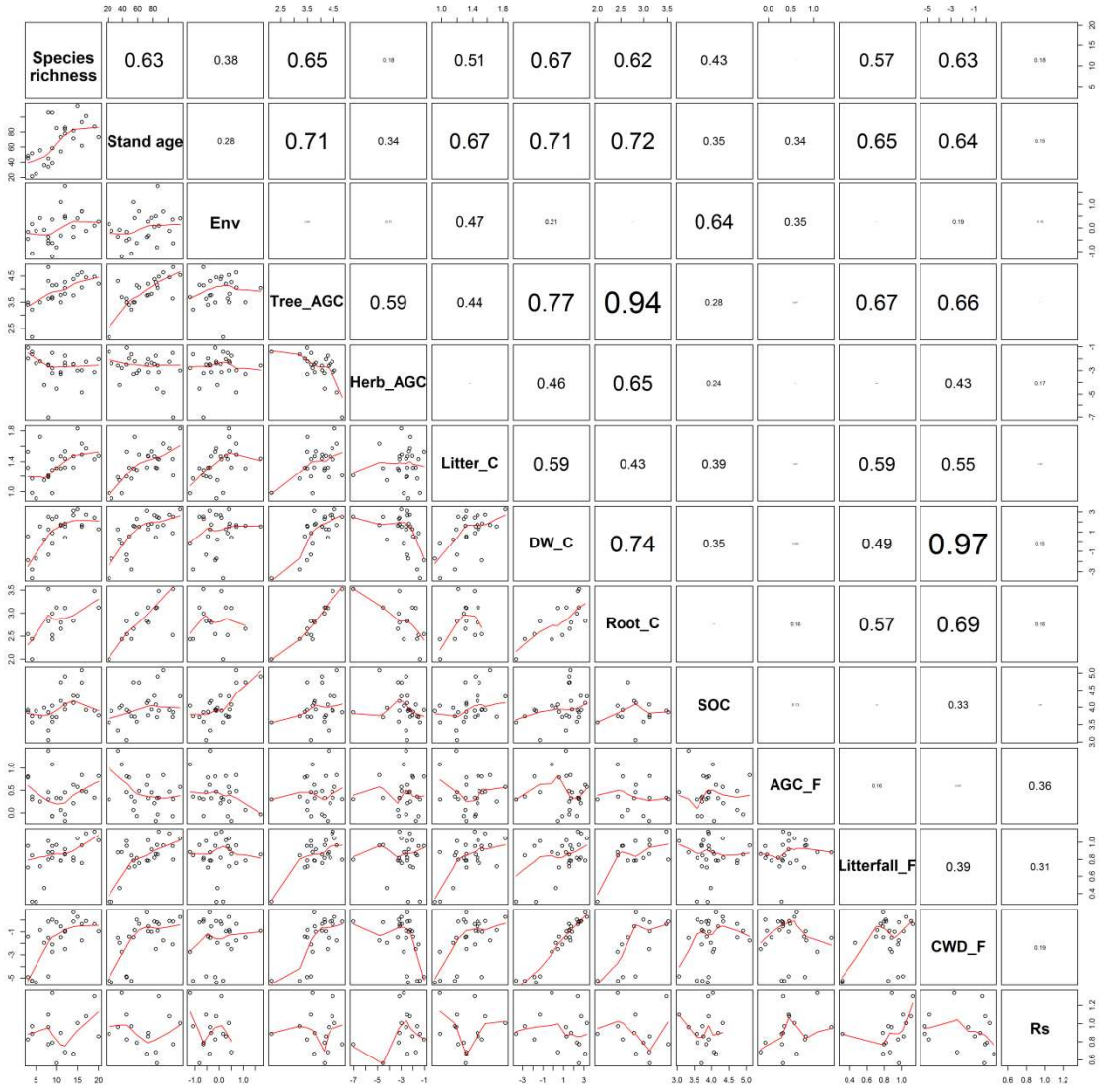

Figure S3. Path analyses illustrating hypothesized causal relationships between variables. a) If considered together, all three explanatory variables, tree species richness (SR), stand age (Age) and environmental variation (environmental PC1, Env), affect aboveground C productivity (AGC_F), but the influence of stand age is negative whereas the influence of tree species richness is positive; b) AGC_F and SR together increase tree aboveground C stocks (Tree_AGC), while SR increases C stocks in roots (Root_C), which is in turn correlated with Tree_AGC; c) and d) indicate that SR effects on litterfall (Litterfall_F and Litter_C) and dead wood variables (DW_C and CWD_F), respectively, are indirect and work via Tree-AGC. Solid arrows indicate positive effects, dashed arrows negative effects; double-headed arrows indicate correlations; values represent standardized coefficients with their significance indicated by asterisks (***: p<0.001, **: p<0.01, *: p<0.05, ns: p>0.05). The overall fit of all models (a–d) was good as indicated by χ2-tests: a) p=0.160, b) p=0.445, c) p=0.793, d) p=0.069.